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CHALKBROOD AND DISEASE CONTROL IN HONEY BEES

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Chalkbrood and Disease Control in Honey Bees

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Final Report: September, 2001
Saskatchewan Agri-food Innovation Fund Grant 97000001

Chalkbrood and Disease Control in Honey Bees

Summary:

The objectives of this project are to investigate the efficacy of the acaricide, formic acid, for control of chalkbrood and *Nosema* disease in honey bees and to estimate the impact of formic acid on the rate of growth of honey bee populations.

Formic acid appeared to have both direct and indirect effects on the development of chalkbrood disease in honey bees but it did not affect the development of nosema disease when applied to colonies as a spring treatment.

Formic acid is highly variable in terms of its efficacy against varroa mites. This study has shown that the level of formic acid found inside the colony varies considerably even when the same application method is used. Temperature and other environmental factors combine to have a major impact on the concentration of formic acid found in hive air. Weight loss of formic acid from treatment devices does not relate to release of acid into the air. Average peak formic acid concentrations in hive during the day air range from about 40 ppm to 100 ppm for the pour on formulation and about 3ppm to 42 ppm for the slow release formulation. The greatest concentrations of formic acid in hive air are found during the early part of the treatment period when slow release formulations are used.

The use of slow release formulations of formic acid was found to have a negative effect on colony development in two out of three years tested. Slow release formulations of formic acid reduced the rate of brood production reducing overall population growth. Therefore, producers should exercise caution when using slow release formulations in the spring.

Under the type of conditions found on the Canadian Prairies where time available for colony population buildup is short, the slow release formulation should only be used if levels of mites exceed the economic threshold and alternative treatments are not available. Under high mite loads colonies treated with formic can perform better than those with high mite loads that are left untreated.

Technical Report

Background and Objectives:

Beekeepers in Saskatchewan who are treating their colonies with menthol and formic acid have noted reductions in the levels of chalkbrood disease in their colonies. A corresponding increase in the levels of chalkbrood, *Ascophaera apis,* and *Nosema apis,* disease have been found in colonies where tracheal mites are at a high level. These observations have led to the speculation that treatments for tracheal mites may be also be beneficial as a prophylactic treatment

for chalkbrood and/or *Nosema* disease. Alternately, the reduced levels of disease found in colonies treated with acaracides may be due to higher levels of "tolerance" to these diseases that are exhibited by "healthier" mite-free bees (Shimanuki et al. 1992). Mite-free bees for example, may have a greater capacity to conduct hygienic behavior that keeps these diseases in check. The causal relationship between acaracide treatment and reductions in diseases needs to be determined so beekeepers can make informed management decisions.

Project Objectives:

The objectives of this project are to investigate the efficacy of the acaricide, formic acid, for control of chalkbrood and *Nosema* disease in honey bees and the impact of formic acid on the rate of growth of honey bee populations.

Project Activities:achievments papers written

Ostermann, D. 2001. Interactions between formic acid, chalkbrood, nosema and *Varroa destructor*. Manitoba Beekeepers Association 91th Annual Meeting. Winnipeg, Manitoba. February.

Currie, R.W. 2001. Use of Formic Acid to Control Varroa Mites in the Wintering Building. Annual Meeting of the Saskatchewan Beekeepers Association. Saskatoon, Saskatchewan. February.

Currie, R.W. 2001. Economic Impact of Varroa Mites: Managing with Formic Acid. Annual Meeting of the Saskatchewan Beekeepers Association. Saskatoon, Saskatchewan. February.

Currie, R.W. 2000. Use of Formic Acid to Control Varroa Mites in the Wintering Building. Annual Meeting of the Alberta Beekeepers Association. Edmonton, Alberta. November, 2000.

Currie, R.W. 2000. Economic Impact of Varroa Mites: Managing with Formic Acid. Annual Meeting of the Alberta Beekeepers Association. Edmonton, Alberta. November, 2000.

Osterman, D. 2000. Interactions between Varroa Mites and Honey Bee Diseases. Manitoba Beekeepers Association 90th Annual Meeting. Brandon, Manitoba. February, 2000.

Currie, R.W. 2000. Varroa Mites: Managing with Formic Acid? Canadian Honey Council Research Symposium. February, 2000. Saskatoon, Saskatchewan

Currie, R.W., 1999. Varroa mites: Are we managing with formic acid? Manitoba Beekeepers Association: 89th Annual Meeting. 9 February, 1999.

Currie, R.W. 1999. Research into chalkbrood control in honey bees. Red River Apiarists

Association. Winnipeg, Manitoba. 8 March, 1999.

Currie, R.W. 1999. Honey bee research at the U. of M. Red River Apiarists Association. Winnipeg, Manitoba. 8 March, 1999.

Currie, R.W. 1999. Manitoba Beekeepers Association: 93rd Annual Meeting. Winnipeg, Manitoba. 6 February, 1996.

Results and Conclusions

Colonies of honey bees containing tracheal and varroa mites were treated with formic acid using different application methods and assessed to determine the potential impact of these treatments on colony development, varroa mite populations, tracheal mite populations, chalkbrood disease and nosema disease. Experiments were repeated on mite-infested and mite-free bees to determine potential interactions between these two "diseases" and the presence or absence of mites. This work was conducted, in conjunction with other experiments designed to determine the impact of formic acid on the varroa mite (Sask Agri-Food Project 970000003).

Summer 1 (1998)

Field Trials

Materials and Methods:

In the spring of 1998, 45 colonies of honey bees were selected from a group of wintered colonies and randomly assigned to each of the following three treatments: 1. Untreated control colonies(n=15); 2. Colonies were treated with 200 ml formic of 65% acid that was placed upon a felt pad contained inside a ziploc bag with slits cut in the plastic ; and 3. Colonies treated with 200 ml formic acid on a felt pad contained inside a ziploc veggie bag®.

For forty two days after treatment all colonies were monitored to measure the bag weight loss.

On 04/06/98 all colonies were sampled to determine the pretreatment levels of varroa mites, tracheal mites, nosema in adult worker bees and to assess the area of sealed brood, and number of adult bees in each colony. Treatments were applied on 05/06/98 and additional samples were taken on 16/06/98, and 29/06/98. Honey production was assessed in the August by removing the honey supers, weighing them and subtracting the net weight of the frames and boxes. Following the removal of the honey supers additional samples of worker bees were collected on 21/08/98 and 29/11/98 to assess the relative abundance of varroa mites, tracheal mites, nosema. Sampling methods used to determine the level of varroa mites, tracheal mites and nosema are described below.

Summer 2 (1999)
Field Trials
Materials and Methods:

Four treatments were applied to 32 nosema- and chalkbrood-infested colonies in the spring. The treatments consisted of: (1) varroa and formic acid, (2) varroa and no formic acid, (3) no varroa and formic acid, and (4) no varroa and no formic acid.

Bees for the 32 colonies came from "strong" hives at the University yard. The bees were shaken from frames, through a funnel, into packages. Each package contained approximately 2 lbs of bees. To create "varroa" and "no varroa" treatments, half of the packages (all 32 packages originally contained known varroa infestation levels), were exposed to fluvalinate (Apistan). The packages were then shaken into standard Langstroth brood chambers, at 4 separate sites (separating the 4 treatments), at the Glenlea Research Station. Each brood chamber contained two frames of honey and two frames of pollen. Each hive also received a pail of feed. Colonies treated with formic acid were initially exposed to the slow-release formulation - 200 mL of 65% formic acid, on a felt pad, in a perforated bag, for a duration of 43 days.

For nosema infestation, the brood-containing frames of each hive was sprayed once with 100 mL of sugar syrup (1 sucrose sugar:1 distilled water by weight) containing 10s *N. apis* spores after Malone et al. (1992). The spores came from bees infected with nosema. The process was as follows: Bees were scooped from infected hives in sampling cups and frozen for about a week. The bees were counted and placed in a pestle with 1 mL distilled water per bee. The mixture was macerated with a mortar. The solid bee parts were filtered from the mixture and a small amount of the solution was taken up by a micropipette. The solution was then placed on a haemocytometer, under a cover slip. The solution was examination under a microscope and the number of (tic-tac like) spores was counted at 400X magnification (Fingler 1978). Nosema infestation was calculated as the number of spores per bee.

For chalkbrood infestation, the broodnest frames of hives were sprayed once with 75 mL of sugar syrup (1 sucrose sugar:1 distilled water) containing three black macerated chalkbrood mummies. This was a variation of Koenig et al. (1987) where the chalkbrood syrup was applied every 6-8 days, repeated 5 times.

The colonies were sampled throughout the spring and fall to assess the levels of nosema, chalkbrood, and varroa infestations, and to assess the impact of treatments on brood area and honey bee abundance.

Nosema samples consisted of approximately 25 bees, taken from the cluster. Samples were prepared and examined as described above. The number of chalkbrood cells and bottom board chalkbrood were counted in each hive. Chalkbrood infestation was calculated as the number of cells containing chalkbrood per cm2 of brood area. Varroa samples consisted of about 100 bees (when hives were at least 3500 bees strong), taken from the cluster. Bees were scooped in a cup and frozen for a week. The bees were counted and placed in a alcohol wash which washed the mites from the bees. The mites were counted and varroa infestation was calculated as the percentage of varroa per bee. Brood area was estimated using a transparent grid. The grid was placed over each frame containing brood and the "area squares" were counted. Brood area was measured in cm2. Honey bee abundance was estimated by comparing pictures of known numbers of bees to each side of each frame of bees. The numbers of bees were then added up to determine the approximate number of bees per hive.

Summer 3 (2000)
Field Trials
Materials and Methods:

Six treatments were applied to 48 chalkbrood-infested colonies in the spring. The chalkbrood infestation procedure followed the method of Koening et al. (1987).

Treatments consisted of: (1) varroa and slow-release formic acid, (2) varroa and pour-on formic acid, (3) varroa and no formic acid, (4) no varroa and slow-release formic acid, (5) no varroa and pour-on formic acid, and (6) no varroa and no formic acid. Slow-release formic acid treatments, followed the method described for the summer of 1999 except "slit" bags (Ziploc bags with 2 vertical slits on each side) were used in place of "veggie bags. For pour-on formic acid treatments, 40 mL of 65% formic acid were poured onto paper towels on the hive top-bars, and were reapplied five times at seven day intervals. Colonies that were infected with mites, "Varroa" treatments, each were innoculated with 45 mites. In, "no varroa" treatments no mites were introduced into these colonies.

The colonies were sampled bi-weekly throughout the spring and fall to assess the levels of chalkbrood, and varroa infestations, and to assess the impact of treatments on brood (worker and drone) area and honey bee abundance (as described above). Hobos were used to measure the temperature (2 probes), and relative humidity within each hive, every 30 minutes. This information was used to assess the affect of environmental conditions on formic acid release in colonies.

Formic acid concentration in hive air was monitored using Drager tubes. Daily trends were assessed by comparing and everyday during the third week (arbitrarily chosen). Intraday trends will be determined by measuring formic acid three times a day (9am, 2 pm, and 7 pm), three days during the third week (arbitrarily chosen).

Supers were weighed before being placed on a colony, and after being removed from a colony in the fall, to assess the affects of treatments on honey production.

Results and Major Conclusions

The Effects of *Varroa* (*Varroa destructor*) and Formic Acid on *Nosema* (*Nosema apis*) and Chalkbrood (*Ascosphaera apis*) in Honey Bee(*Apis mellifera*) Colonies

- Formic acid appears to reduce Chalkbrood infection in some situations. Lower levels of Chalkbrood disease were found in colonies treated with FA in two of the three years.

- *Varroa* may act as a vector to increase the level of Chalkbrood in the hive.

- Weight loss from slow release bags containing formic acid is relatively consistent throughout the treatment period. The two types of bags we tested, the slit bag and the "veggie bag" had similar release rates that averaged and gm per day respectively. However, the number of grams lost from the bags, a common measure used to calculate release rates does not relate

to the amount found in hive air.

- Ambient conditions may influence formic acid release rates in the hive. -the correlation between ambient temperature and formic acid release throughout the hive was not significant; ambient temp likely acts in combination with other factors, such as relative humidity and colony strength, and the daily and seasonal pattern of temperatures which act in concert to influence FA release in the hive. Generally higher temperatures are associated with higher release rates into the air but the timing of when those temperatures occur during the day is critical to determining the amount of formic acid that gets into the air. For a pour-on formulation most of the formic acid dissipates within 24 hours but the peak level in hive air can vary from an average of 40 ppm to 100 ppm. For slow release formulations the amount of formic acid release is also related to temperature and the amount in air is typically much lower than that found with the pour-on formulation. High temperatures early in the treatment period will result in the greatest concentrations of formic acid in the hive air. Average peak formic acid concentrations in hive during the day air range from about 3ppm to 42 ppm for the slow release formulation. Although the weight loss from the bags is constant throughout the treatment period the amount of acid volatilised is not. Late in the treatment period high temperatures no longer volatilize as much acid, likely because the formic acid to water ratio does not remain consistent throughout the treatment period.

- Spring treatments with formic acid slow release formulations had a negative impact on colony brood production and adult worker populations in two of the three years studied and a neutral to positive benefit in the other year. However, the use of these formulations mites are present can increase colony population relative to untreated colonies. If mites are present at levels above the economic threshold and alternative controls (such as Apistan) are not available then treatment with a slow release formulation would be justified.

- The potential for *Varroa* to act as a vector of *Nosema* depends *Nosema* infects haemocytes in adult bees and/or can be vectored into larvae and pupae. We found no evidence in this study to indicate that *Varroa* might vector *Nosema*. The application of formic acid to colonies in spring treatments did increase or decrease the level of *Nosema* infection.

- In all three years of our study the level of *Nosema* was relatively consistent between colonies and followed what would be considered to be normal seasonal trends for this disease.

Indications for Further Development

This project has demonstrated that in some years slow release formic acid applications in the spring can hamper colony growth and development which, in turn will reduce honey production. Producers can save money by selecting alternatives control methods when is necessary to treat for varroa mites in the spring. We found that the release rate of formic acid into hive air is not consistent and this variability in release rates under different

conditions creates high levels of variability in the efficacy of control. There is a need to develop a formic acid release device that works reliably under the wide range of environmental conditions that are found in the prairie provinces.

Producers that do use formic acid for control of varroa or tracheal mites may see some additional benefits in the form of reduced chalkbrood levels in their hives in some years. Formic acid appears to reduce chalk brood both indirectly by reducing the populations of varroa mites that vector the disease and directly through the action of acid against the disease organism.

Data from all three years of this study are appended to the back of this report.

Personnel

The following personnel were involved in this project. David Ostermann, M.Sc. Student, Linda Klymochko, technician, Kristina Pohl, summer research assistant, Robyn Underwood, Technician.

Equipment

No major equipment was purchased for this project.

Project Photos

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The Saskatchewan Agri-Food Innovation Fund has been acknowledged in all oral and written presentations made on this research. It will also be acknowledged in all resulting scientific publications.

Expense Statement

Final expense statements have been previously submitted.

Figure 1: Daily weight loss of formic acid from two different types of slow release formulations. Slit bag=ziploc freezer bag containing 200 ml of 65% formic acid on adsorbent boot felt in a bag with three slits cut through the plastic. Veggie bag=ziploc vegetable bag (perforated with holes) containing 200 ml of 65% formic acid on adsorbent boot felt within the bag.

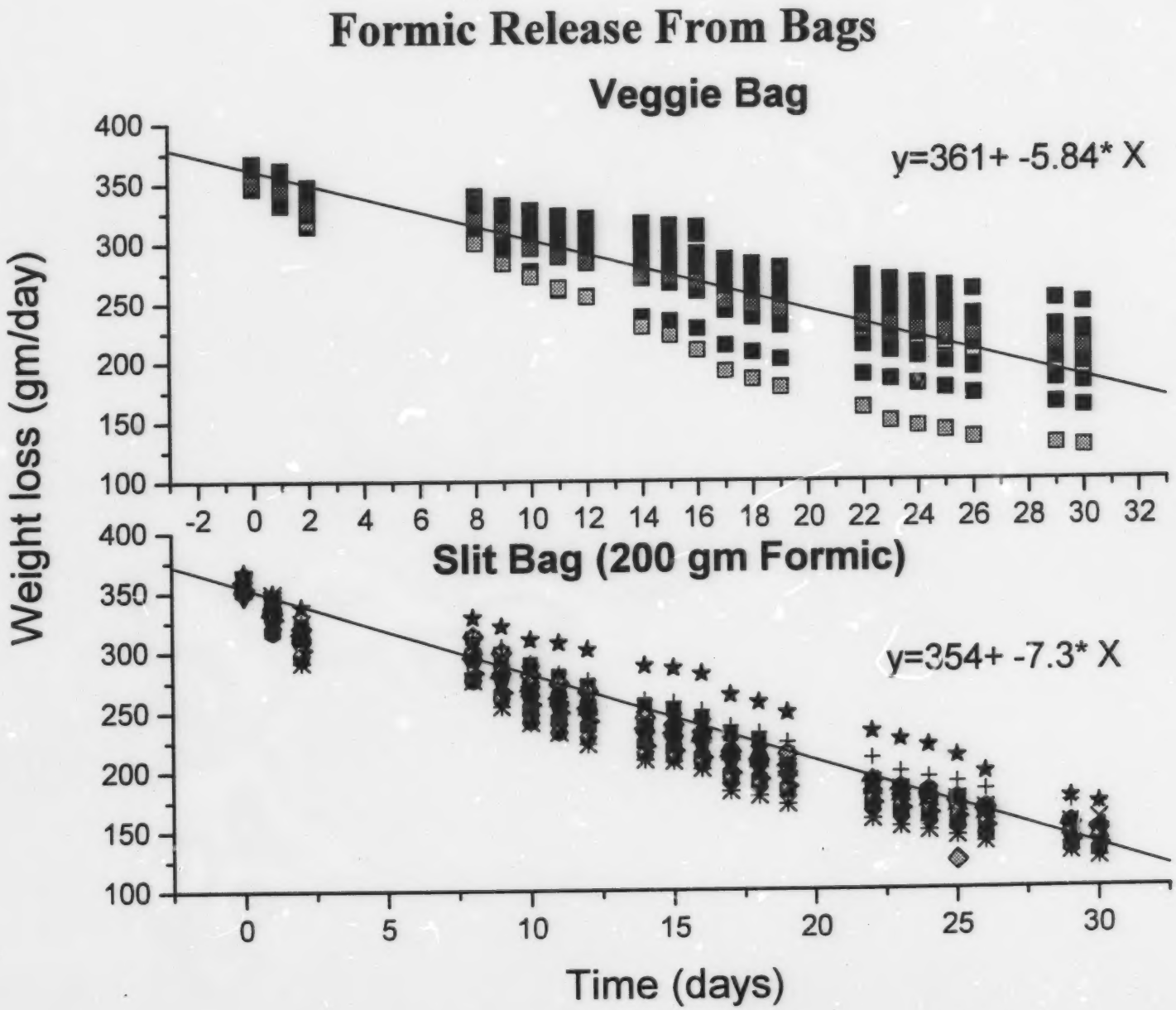
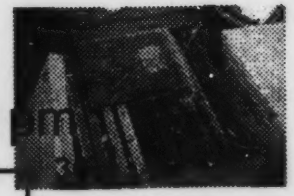

Formic Release From Bags
Veggie Bag
y=361+ -5.84* X
Slit Bag (200 gm Formic)
y=354+ -7.3* X
Weight loss (gm/day)
Time (days)
400
350
300
250
200
150
100
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
5
15
25

Figure 2: Formic acid concentration in hive air (ppm) sampled at 2pm throughout the treatment period. Solid dots indicate ambient temperatures. Pour on samples were collected on the day of treatment, and during mid week. Five pour-on treatments were applied at 7 day intervals. Slow release formulations were slit ziploc bags containing 200 ml of 65% formic acid.



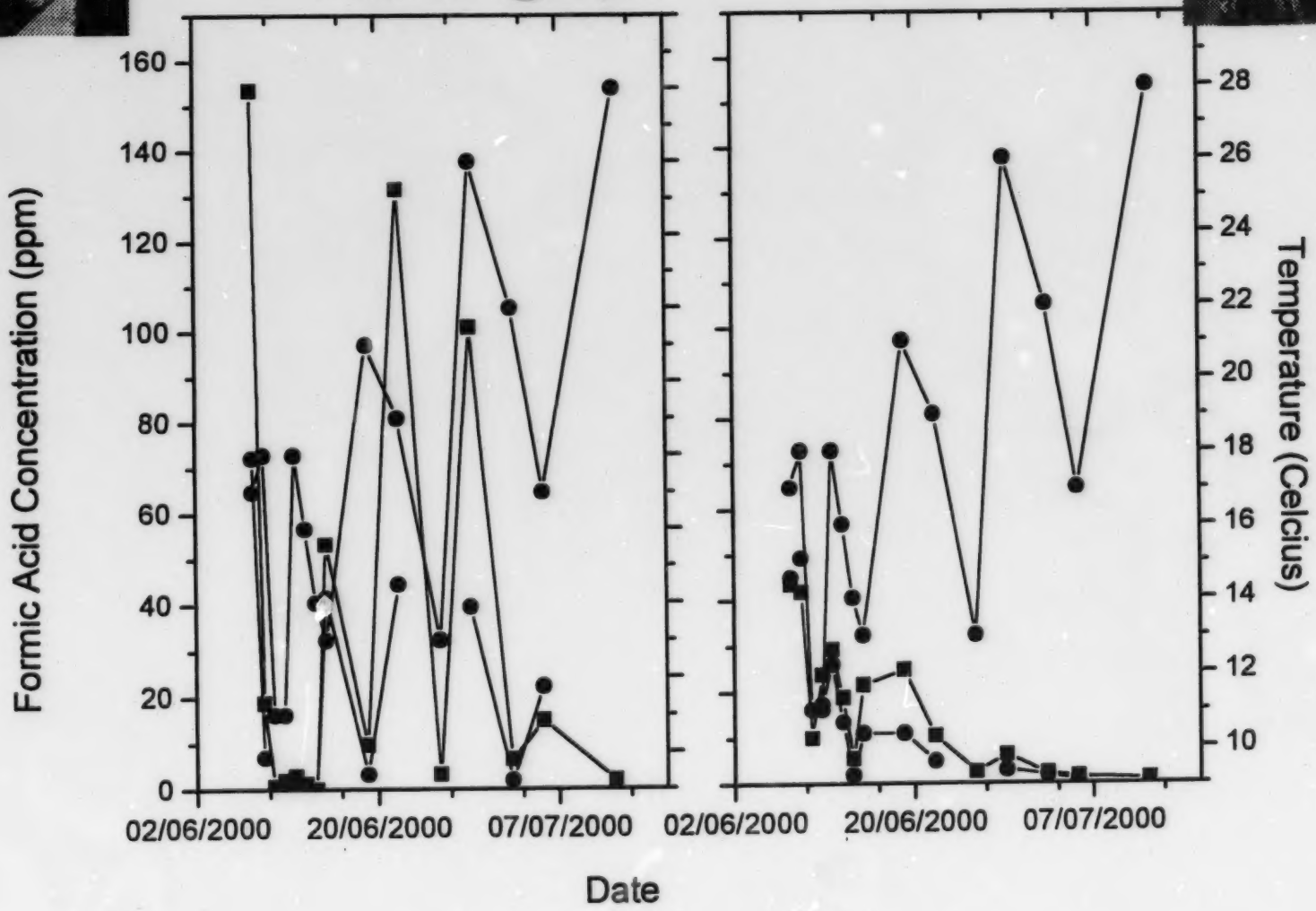

Pour on @ 2 pm
Slow Release @ 2
Formic Acid Concentration (ppm)
Temperature (Celcius)
0
20
40
60
80
100
120
140
160
10
12
14
16
18
20
22
24
26
28
02/06/2000
20/06/2000
07/07/2000
02/06/2000
20/06/2000
07/07/2000
Date

Figure 3: Within-day levels of formic acid in hive air (ppm) for pour-on treatments. Solid dots represent ambient temperature. Shaded dots and squares indicate mean values for two sets of treatments. Sampling was repeated on three dates throughout the summer.

Pour On Formulation

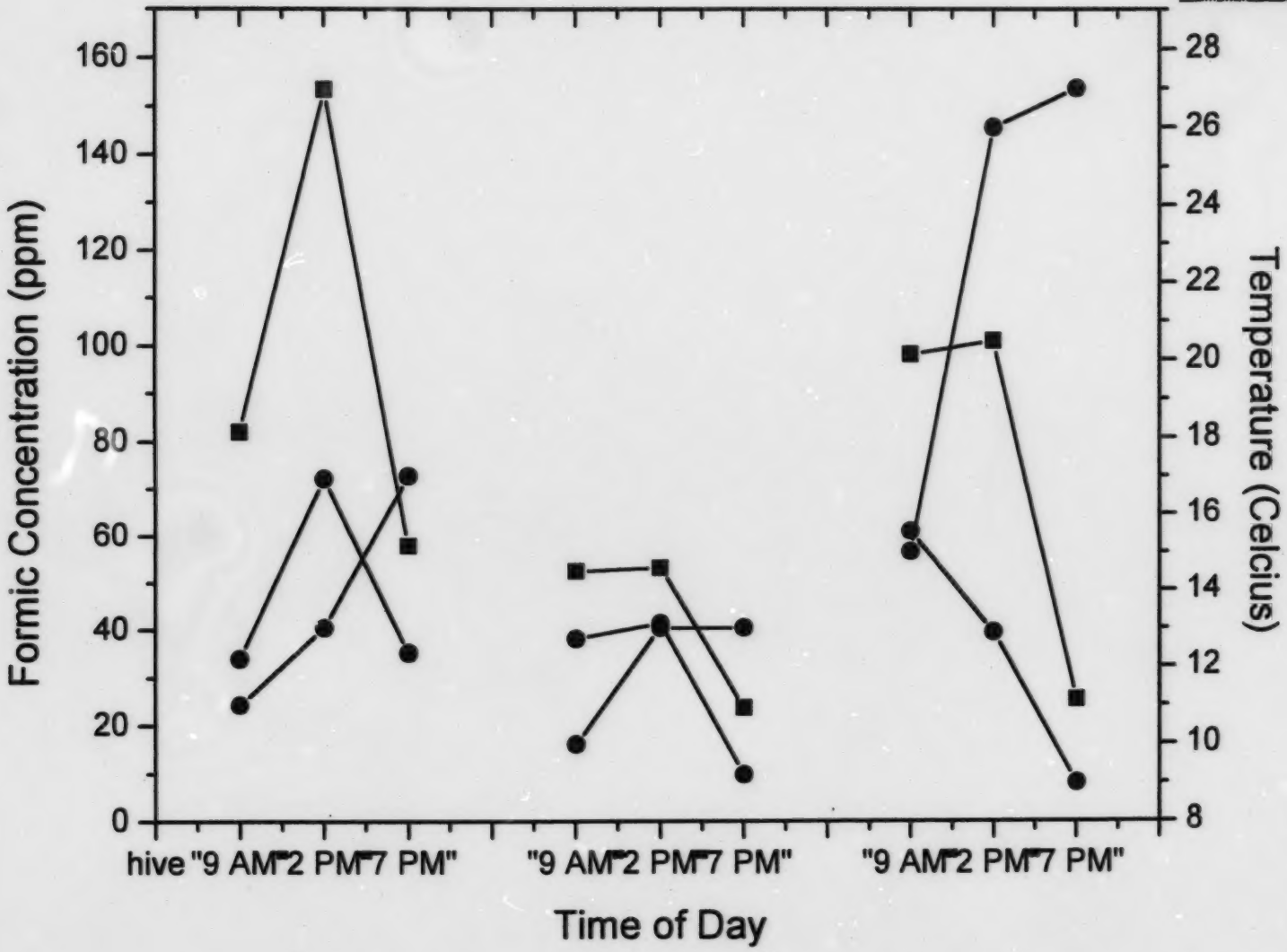


Figure 4: Within day levels of formic acid in hive air (ppm) for slow release treatments. Solid dots represent ambient temperature. Shaded dots and squares indicate mean values for two sets of treatments. Sampling was repeated on three dates throughout the summer.

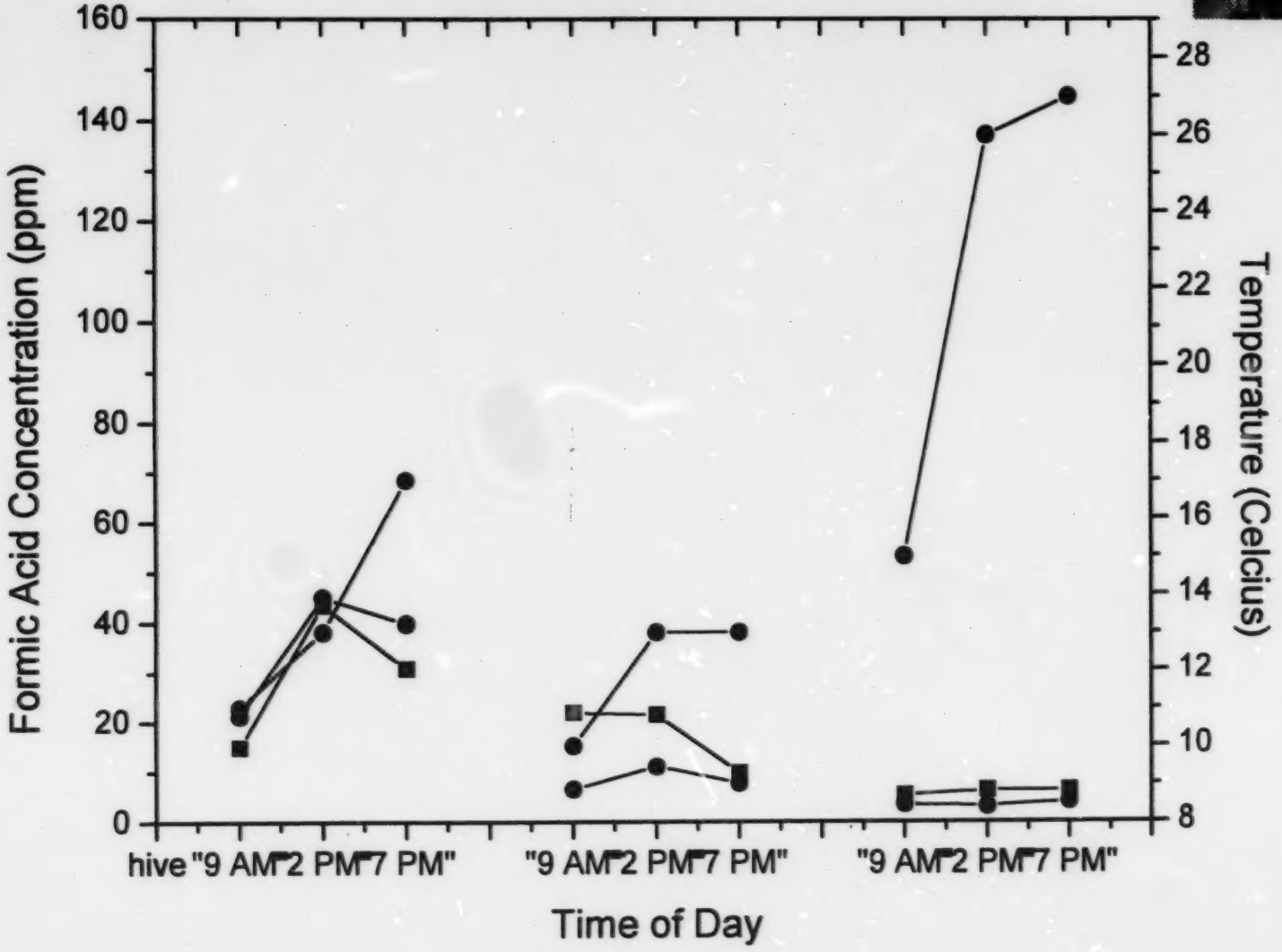

Slow Release Formic
Formic Acid Concentration (ppm)
Temperature (Celcius)
160
140
120
100
80
60
40
20
0
28
26
24
22
20
18
16
14
12
10
8
hive "9 AM" "2 PM" "7 PM"
"9 AM" "2 PM" "7 PM"
"9 AM" "2 PM" "7 PM"
Time of Day

Figure 5: Correlation of formic acid concentration (ppm) in air with solar radiation for the pour-on formulation. Upper line indicates solar radiation level.

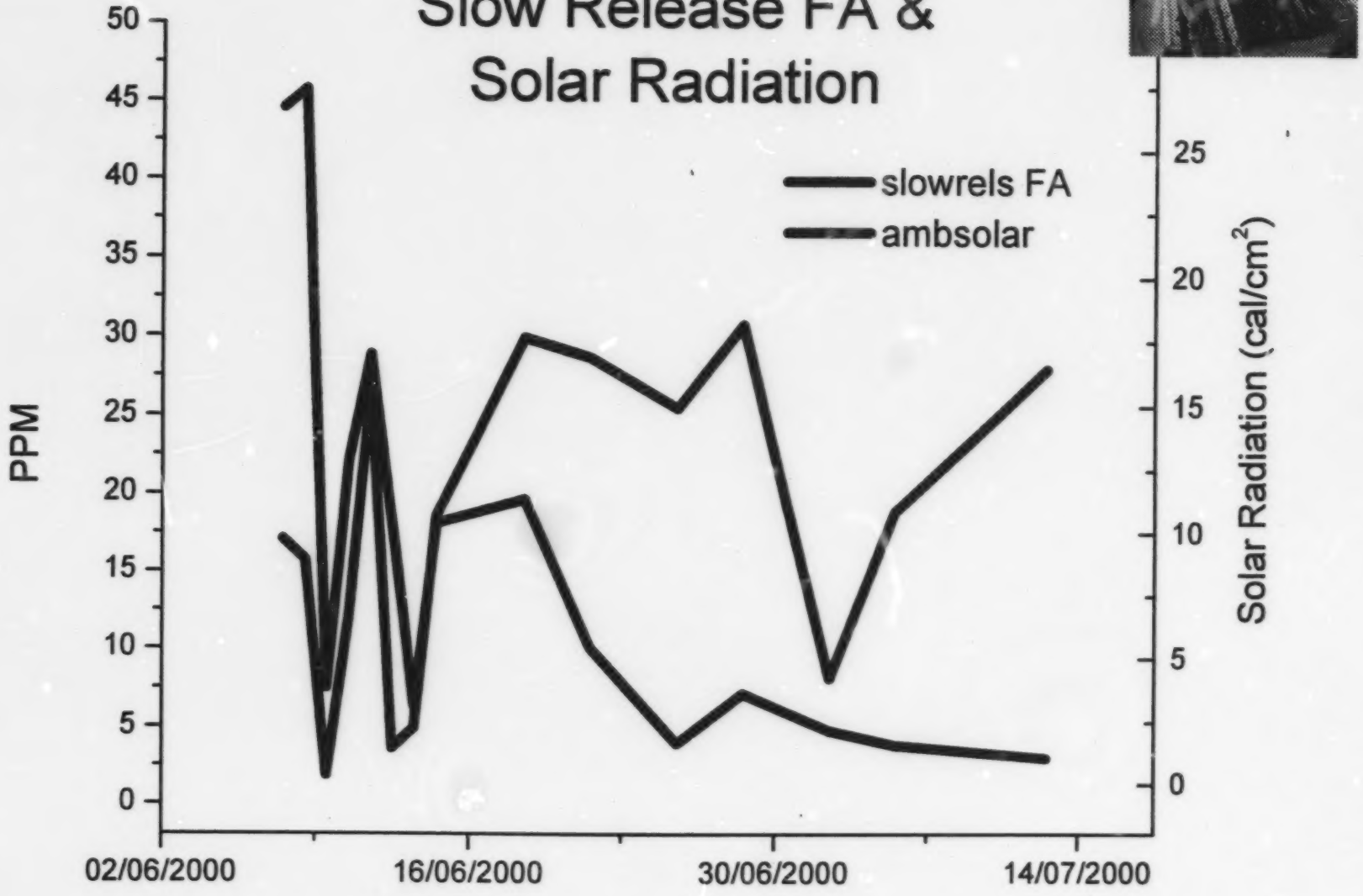

Slow Release FA &
Solar Radiation
slowrels FA
ambsolar
PPM
Solar Radiation (cal/cm²)
50
45
40
35
30
25
20
15
10
5
0
25
20
15
10
5
0
02/06/2000
16/06/2000
30/06/2000
14/07/2000

Figure 6: Correlation of formic acid concentration (ppm) in air with solar radiation for the pour-on formulation. Upper line indicates solar radiation level.

Pour On FA & Solar Radiation

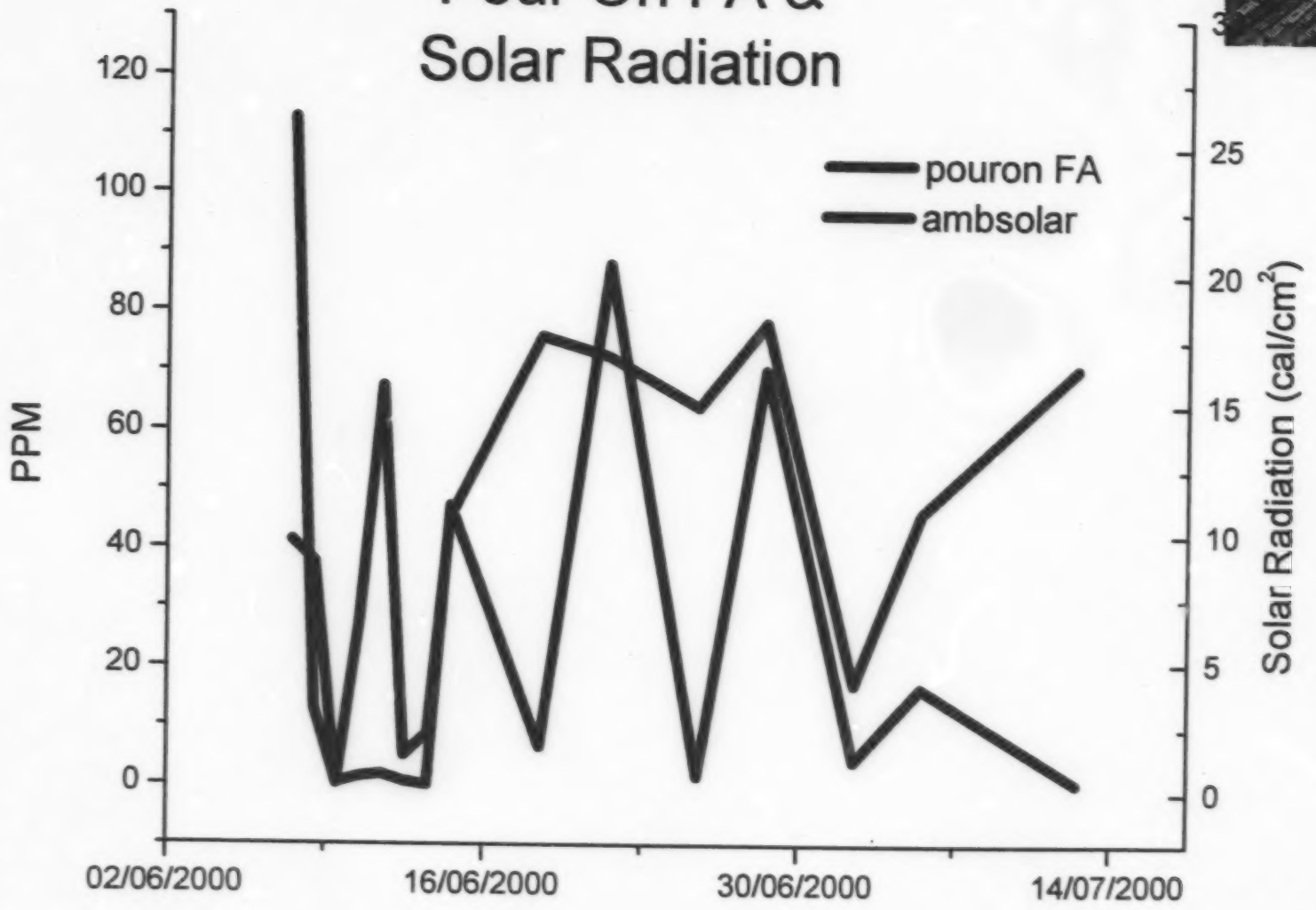
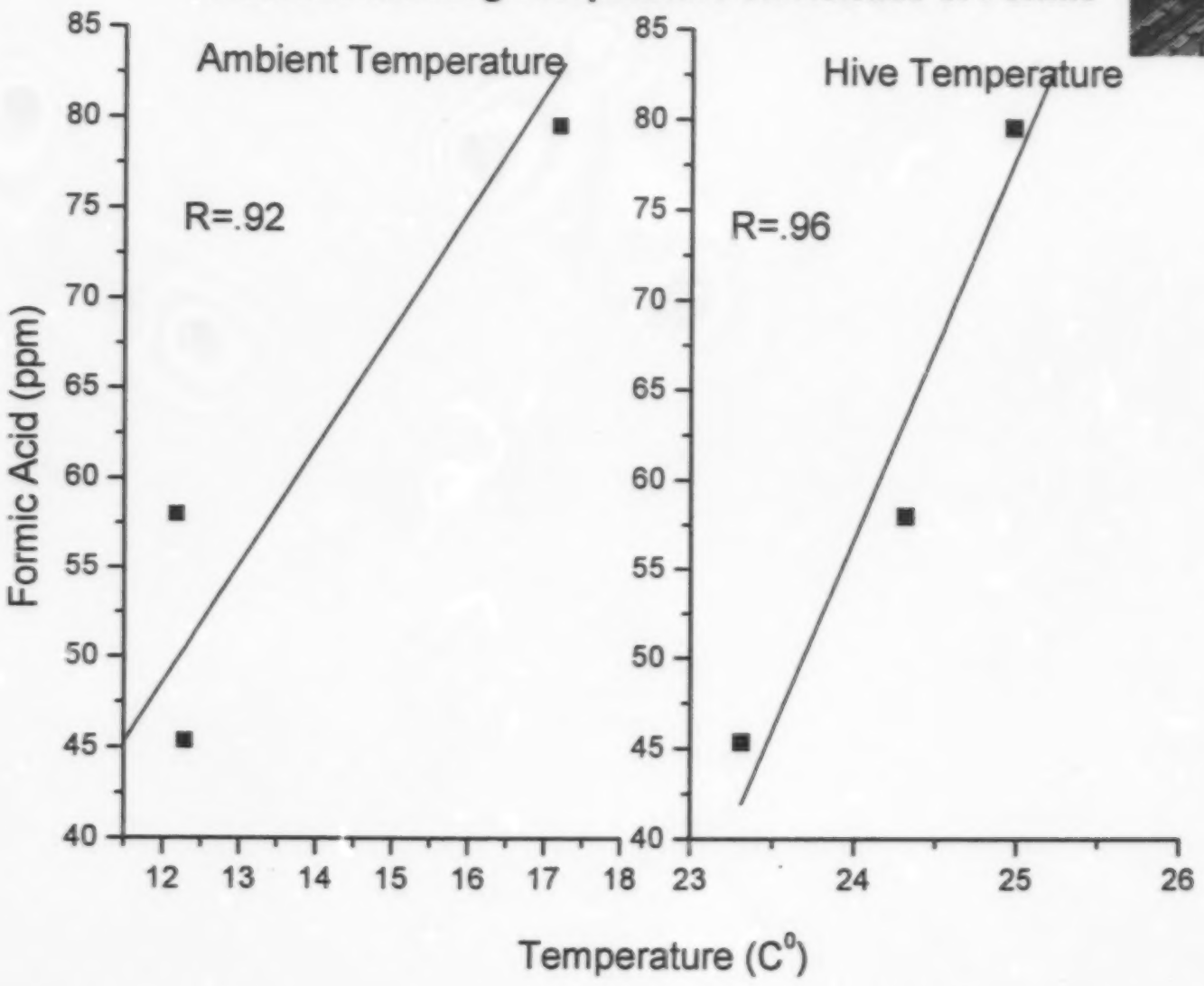


Figure 7: Correlation of formic acid concentration (ppm) in air with morning ambient temperature and hive temperature.


Effect of Morning Temperature on Release of Formic
Ambient Temperature
R=.92
Hive Temperature
R=.96
Formic Acid (ppm)
85
80
75
70
65
60
55
50
45
40
12
13
14
15
16
17
18
23
24
25
26
Temperature (C⁰)

Effect of Afternoon Temperature on Release of Pour on Formic

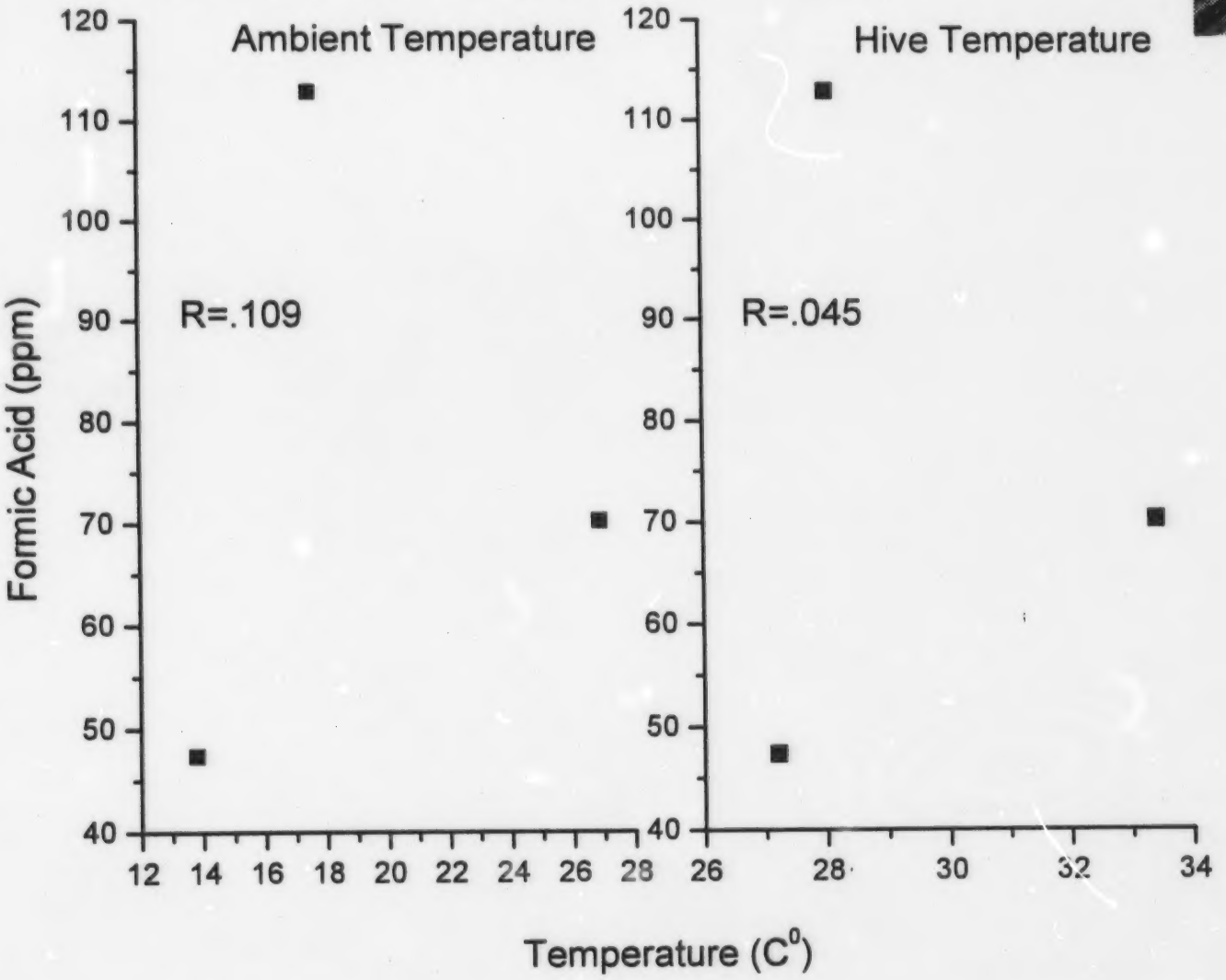


Figure 8: Lack of correlation of formic acid concentration (ppm) in air with afternoon ambient temperature and hive temperature.

Figure 9: Effect of mornings temperature on the correlation of formic acid concentration (ppm) in air with afternoon ambient temperature and hive temperature.

Effect of Afternoon Temperature on Release of Pour on Formic

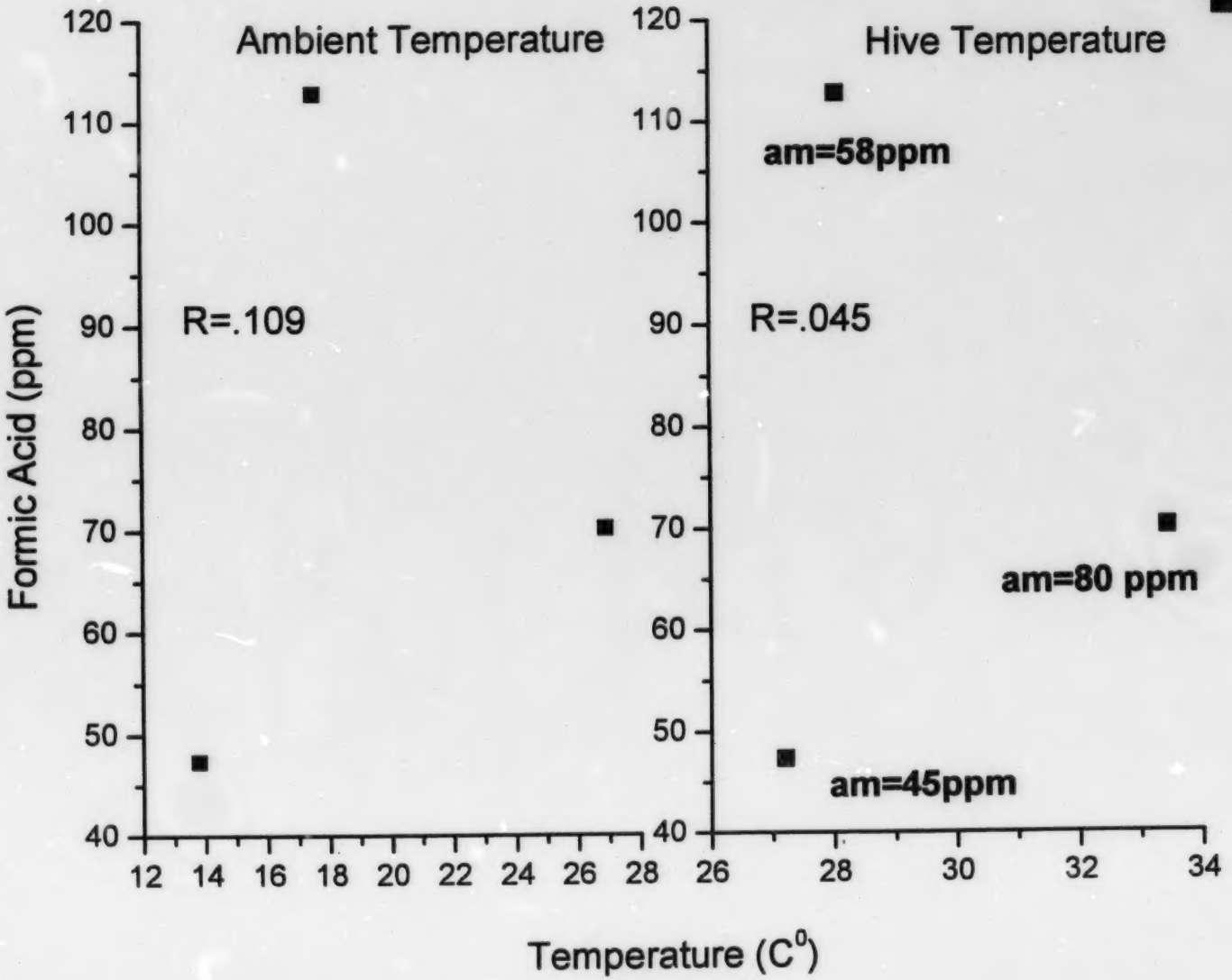


Figure 10: Effecf of two types of slow release treatment and no treatment on the mean number of worker bees and mean number of drones per hive in 1998.

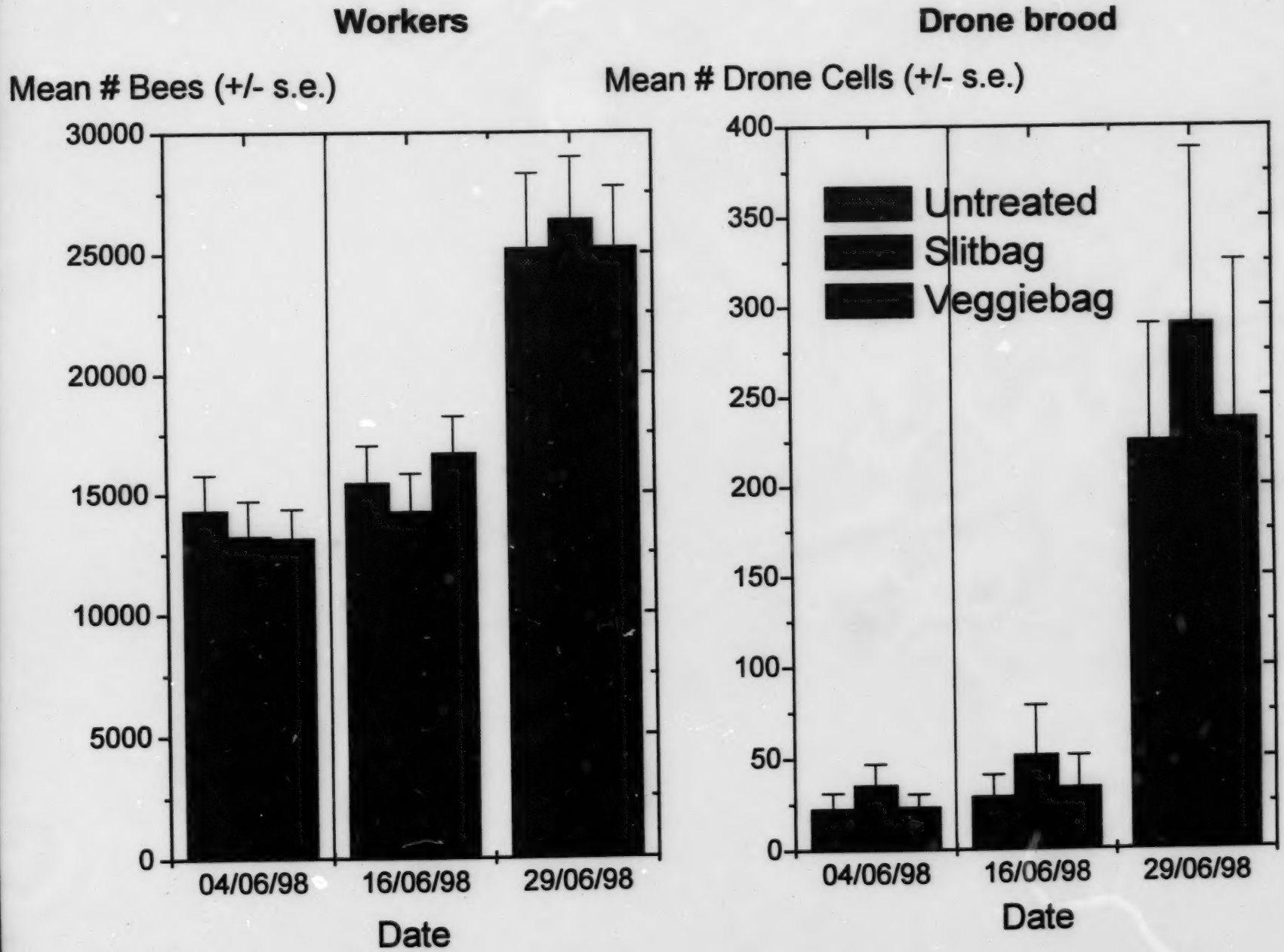

Workers
Mean # Bees (+/- s.e.)
30000
25000
20000
15000
10000
5000
0
04/06/98
16/06/98
29/06/98
Date
Drone brood
Mean # Drone Cells (+/- s.e.)
400
350
300
250
200
150
100
50
0
Untreated
Slitbag
Veggiebag
04/06/98
16/06/98
29/06/98
Date

Figure 11: Effect of spring slow-release formic acid treatment on brood production in 1999. FA = formic acid treatment. NoFA= colonies left untreated

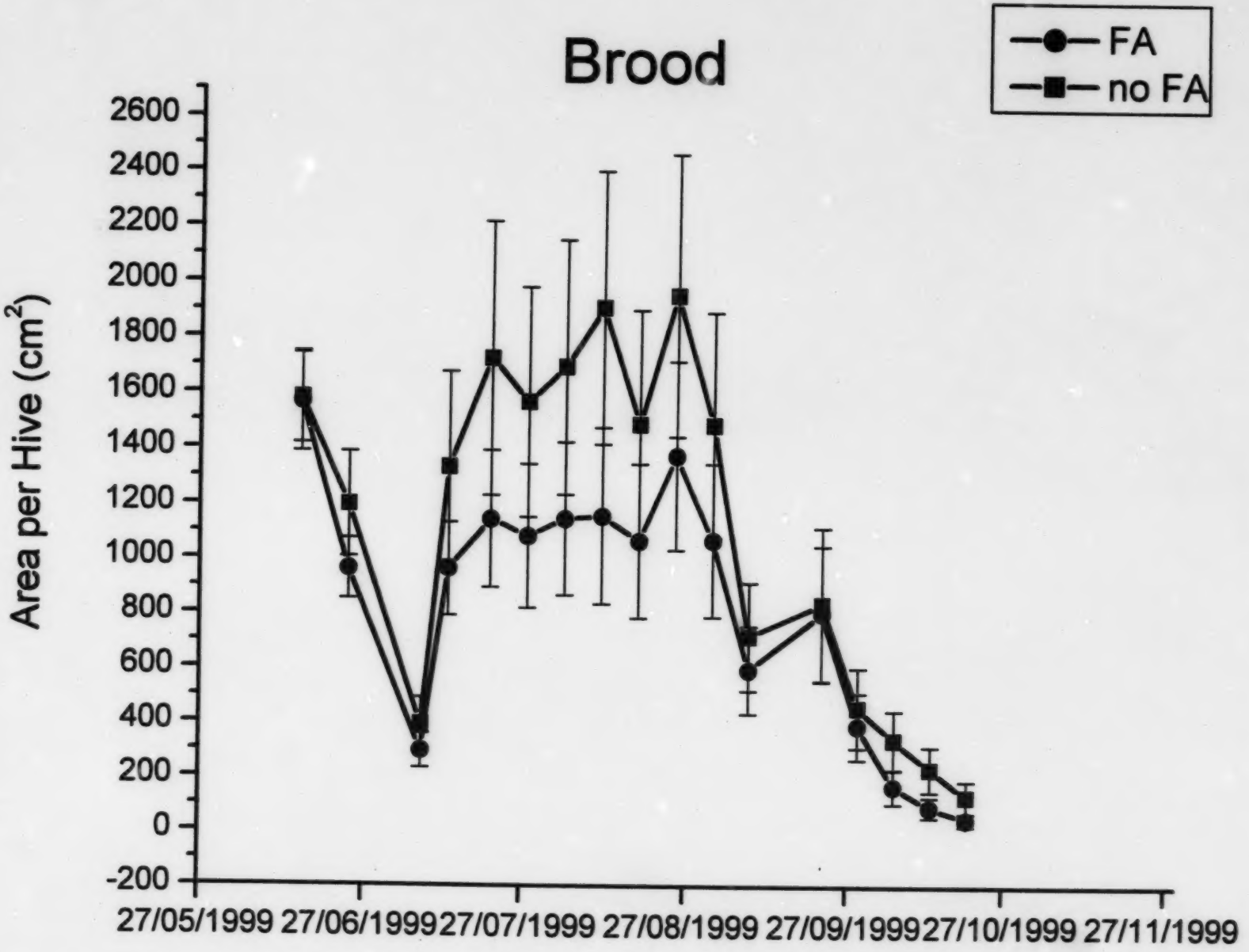

Brood
FA
no FA
Area per Hive (cm²)
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
27/05/1999
27/06/1999
27/07/1999
27/08/1999
27/09/1999
27/10/1999
27/11/1999

Figure 12: Effect of spring slow-release formic acid treatment on worker population in the absence of mites in 1999.. FA = formic acid treatment. NoFA= colonies left untreated

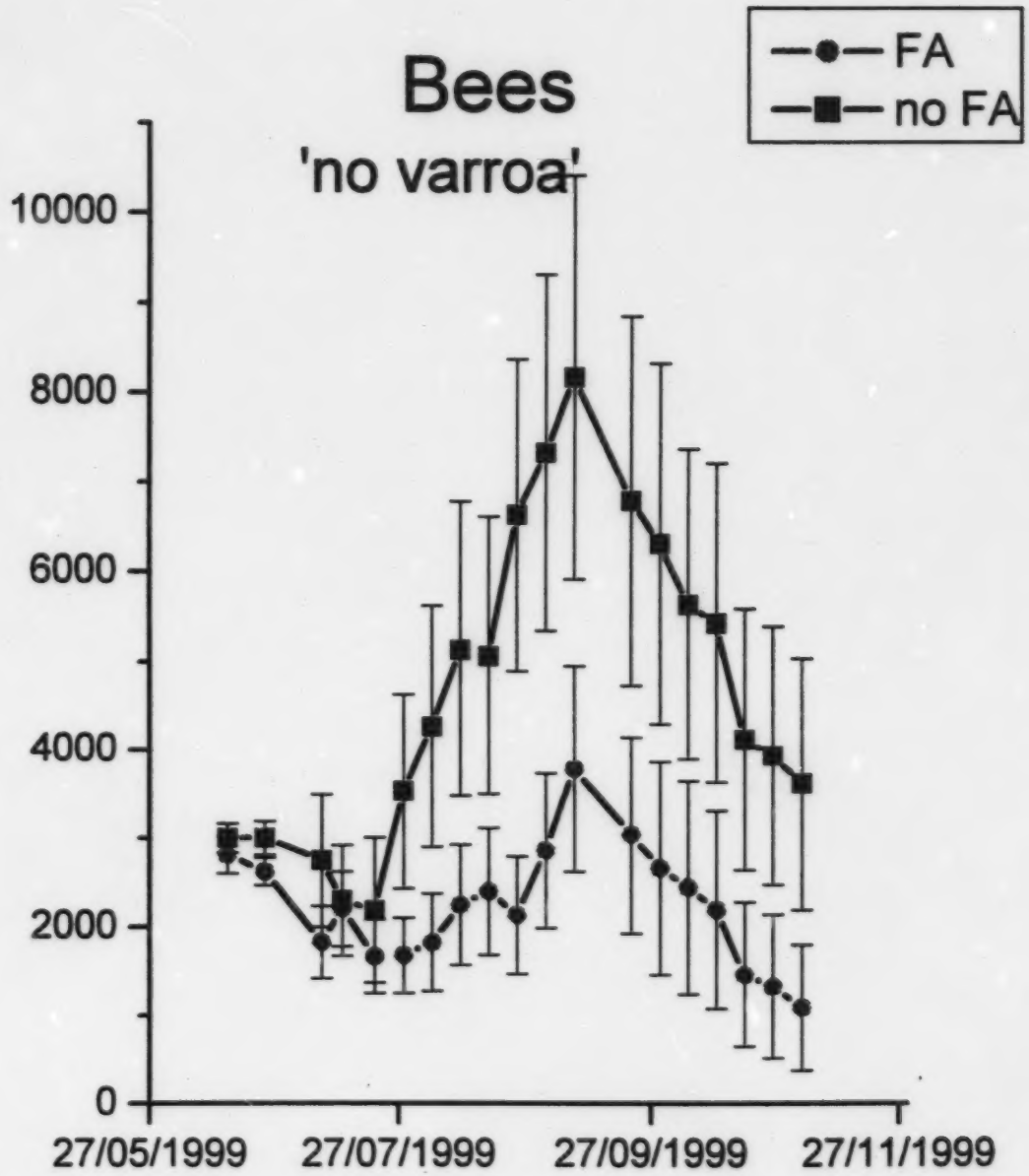

Bees
'no varroa'
FA
no FA
10000
8000
6000
4000
2000
0
27/05/1999
27/07/1999
27/09/1999
27/11/1999

Figure 13: Effect of spring slow-release formic acid treatment on worker population in the presence of mites in 1999.. FA = formic acid treatment. NoFA= colonies left untreated

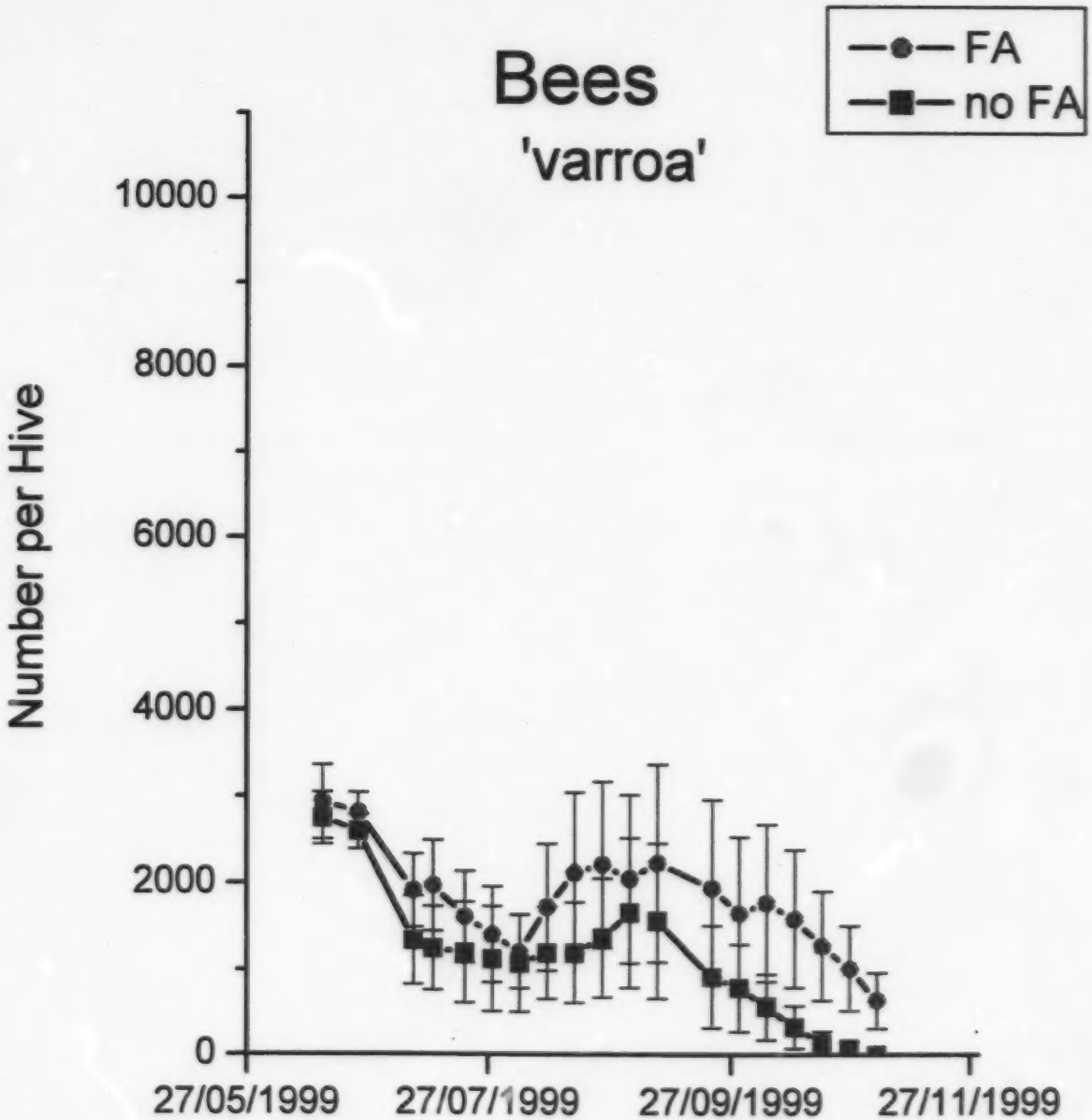

Bees
'varroa'
FA
no FA
Number per Hive
10000
8000
6000
4000
2000
0
27/05/1999
27/07/1999
27/09/1999
27/11/1999

Figure 14: Effect of two types of formic acid treatment and no treatment on the mean number of worker bees and mean number of drones per hive in 2000, when mites were present, or absent.

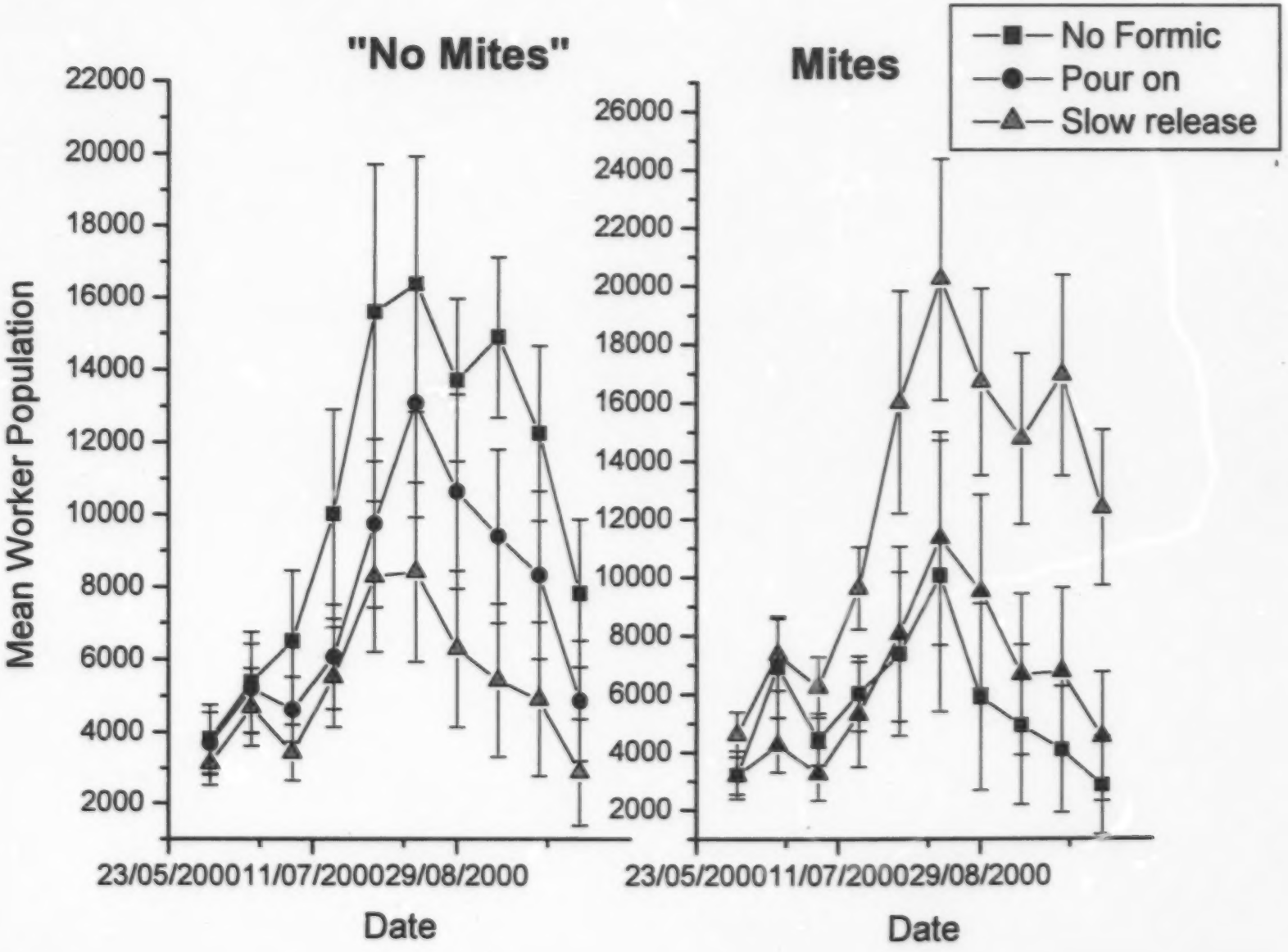

"No Mites"
Mites
No Formic
Pour on
Slow release
Mean Worker Population
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
23/05/2000
11/07/2000
29/08/2000
Date
23/05/2000
11/07/2000
29/08/2000
Date

Figure 15: Effect of two types of slow release treatment and no treatment on the mean number of cells of chalkbrood per hive in 1998. 04/06/98 = pretreatment levels. 16/06/98 and 29/06/98 = post treatment levels.

Chalkbrood

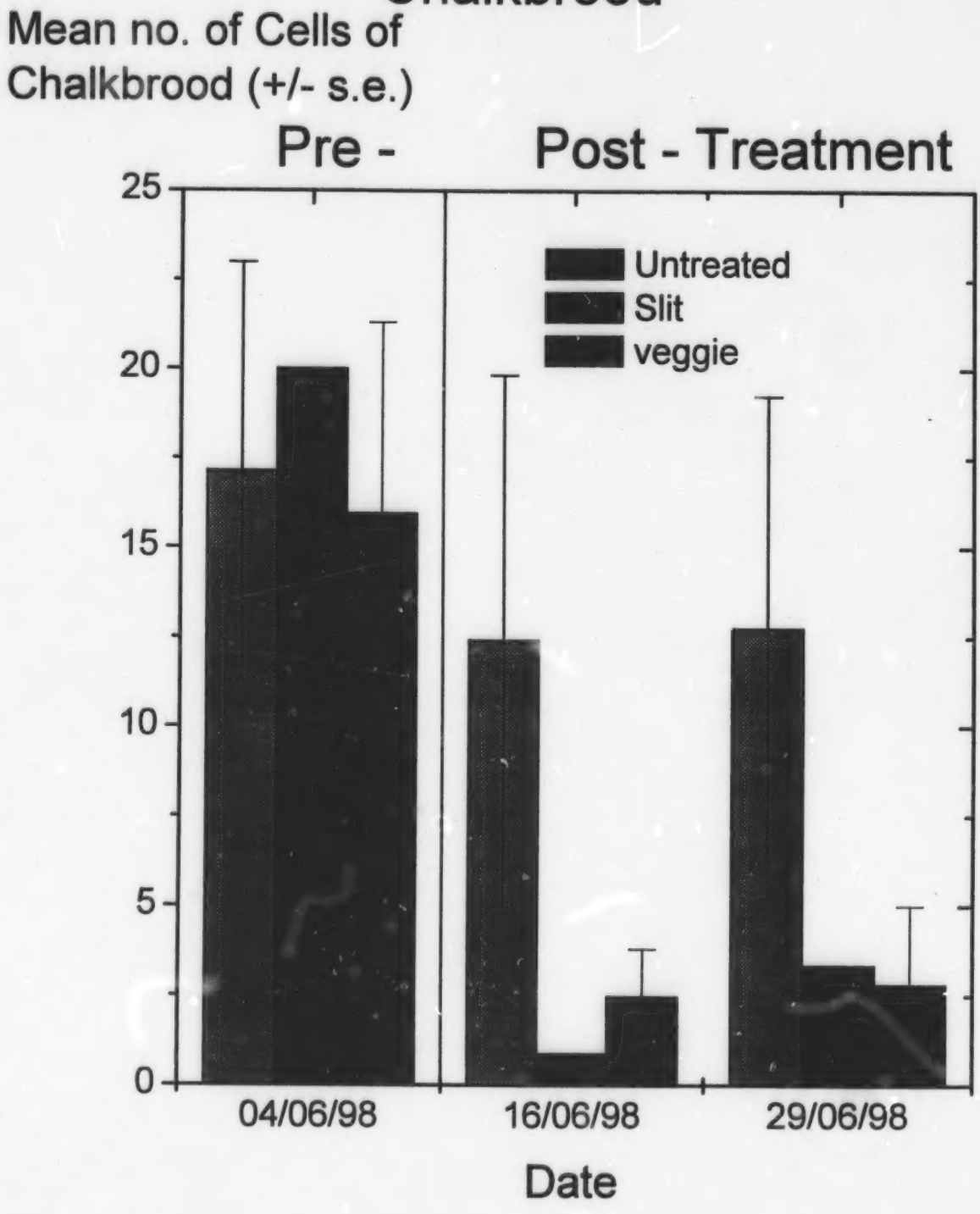

Mean no. of Cells of Chalkbrood (+/- s.e.)
Pre -
Post - Treatment
25
20
15
10
5
0
Untreated
Slit
veggie
04/06/98
16/06/98
29/06/98
Date

Figure 16: Effect of spring slow-release formic acid treatment on chalbrood in the absence of mites in 1999. FA = formic acid treatment. NoFA= colonies left untreated

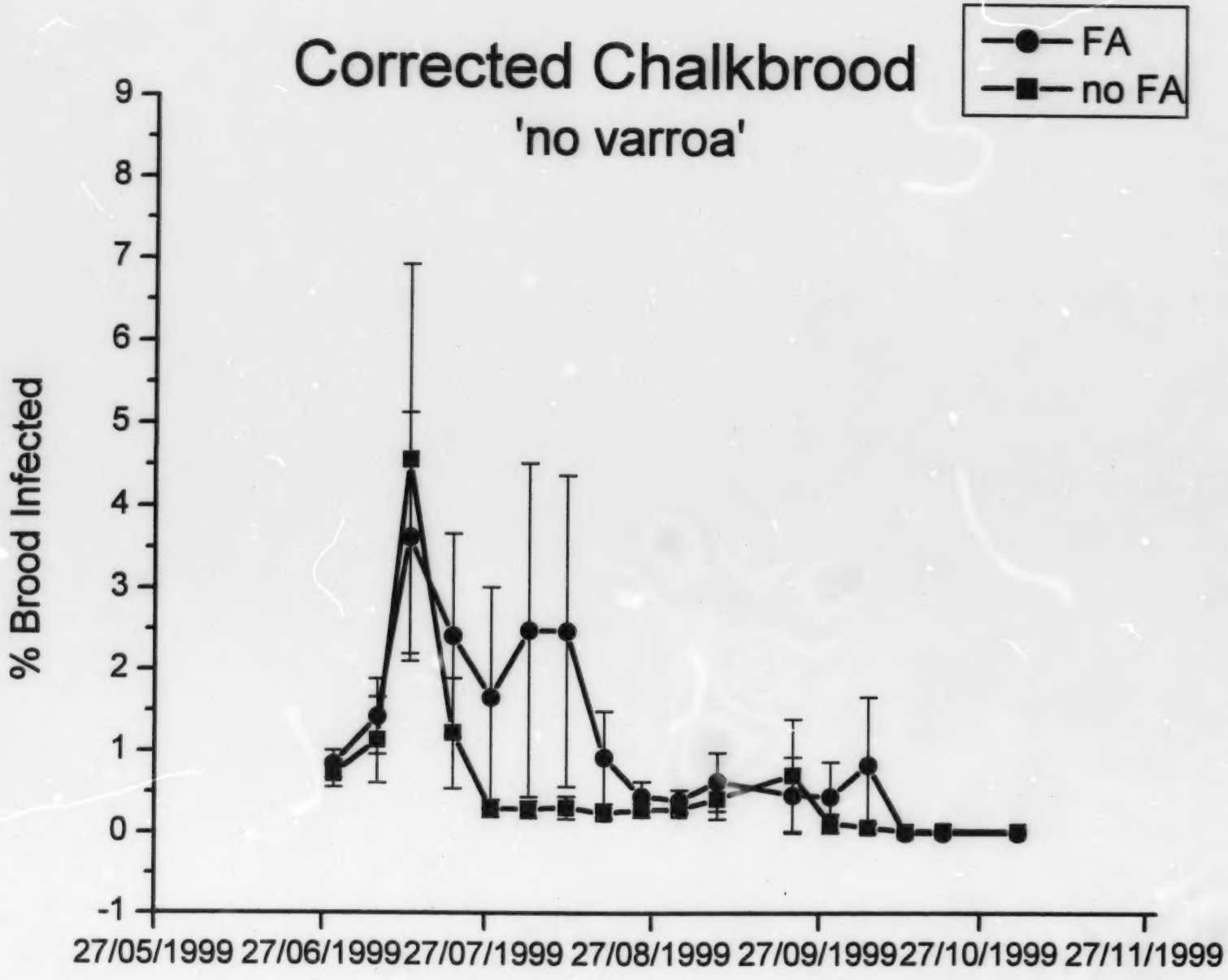

Corrected Chalkbrood
'no varroa'
FA
no FA
% Brood Infected
9
8
7
6
5
4
3
2
1
0
-1
27/05/1999
27/06/1999
27/07/1999
27/08/1999
27/09/1999
27/10/1999
27/11/1999

Figure 17: Effect of spring slow-release formic acid treatment on chalkbrood in the presence of mites in 1999. FA = formic acid treatment. NoFA= colonies left untreated

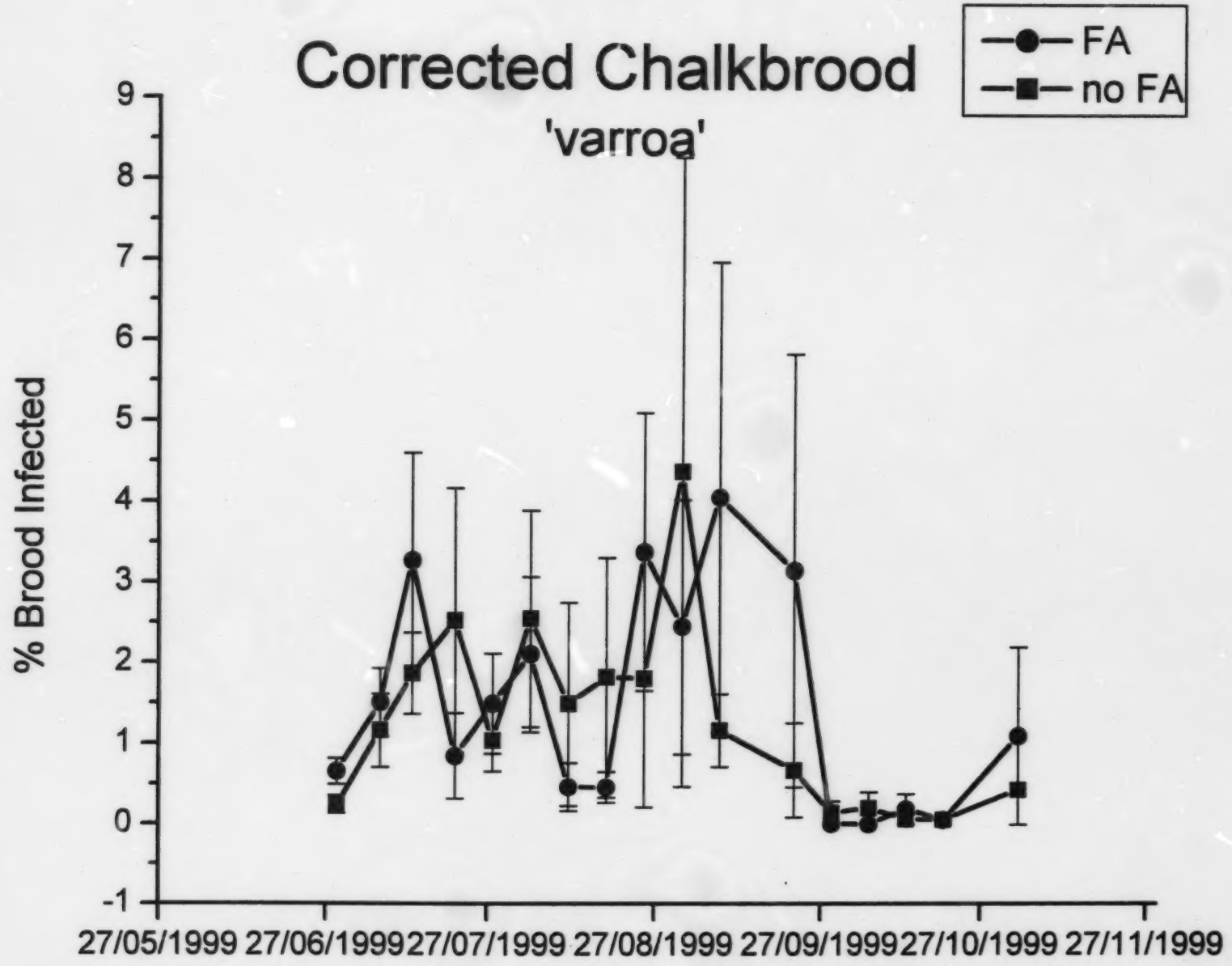

Corrected Chalkbrood
'varroa'
FA
no FA
% Brood Infected
9
8
7
6
5
4
3
2
1
0
-1
27/05/1999
27/06/1999
27/07/1999
27/08/1999
27/09/1999
27/10/1999
27/11/1999

Figure 18: Effect of two types of formic acid treatment and no treatment on the mean percentage of brood cells infested with chalkbrood disease in 2000, when mites were present, or absent.

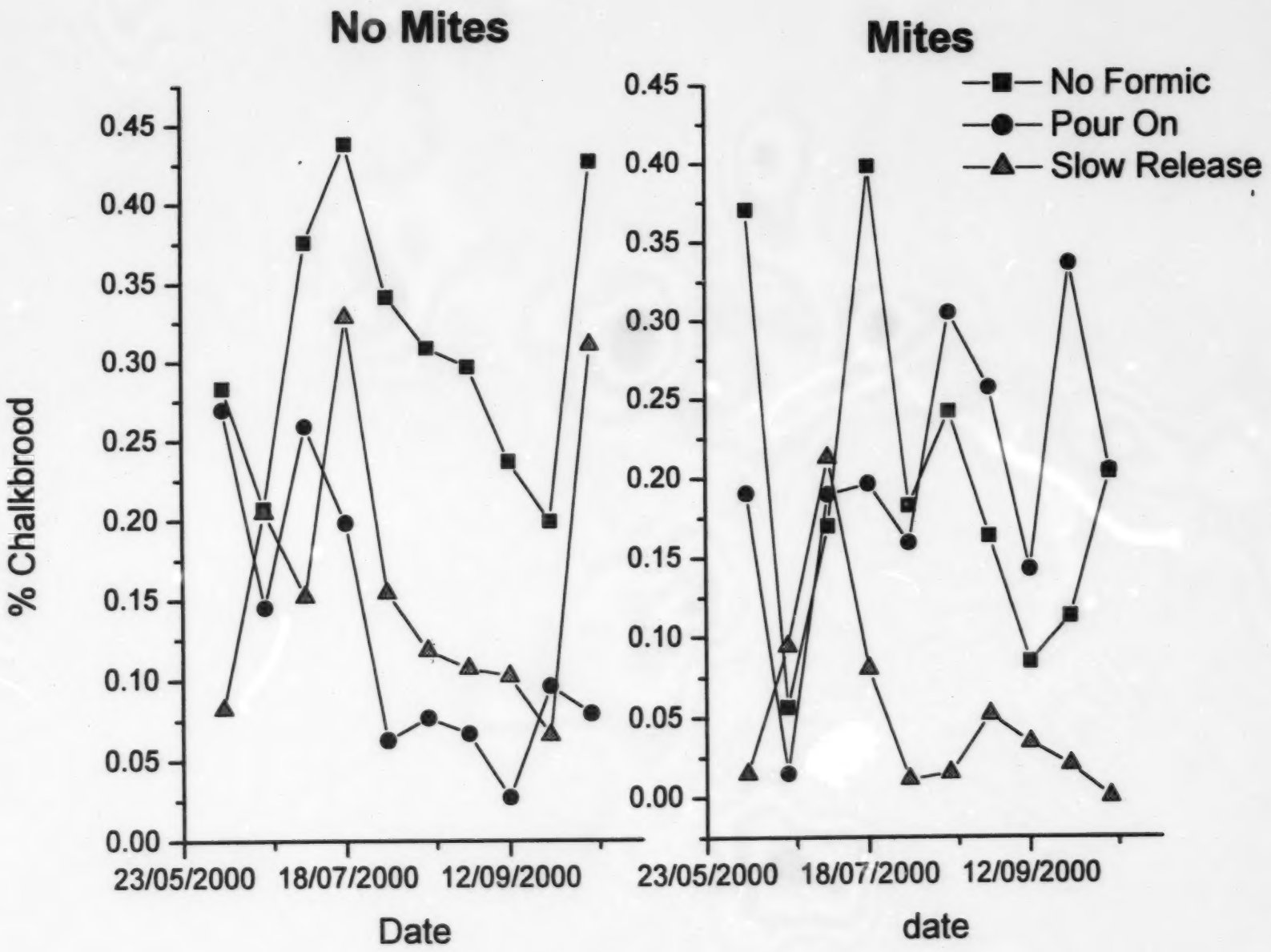

No Mites
Mites
No Formic
Pour On
Slow Release
% Chalkbrood
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
23/05/2000
18/07/2000
12/09/2000
Date
date

Figure 19: Effect of two types of slow release treatment and no treatment on the mean nosema infestation per hive in 1998. 04/06/98 = pretreatment levels. 16/06/98 and 29/06/98 = post treatment levels.

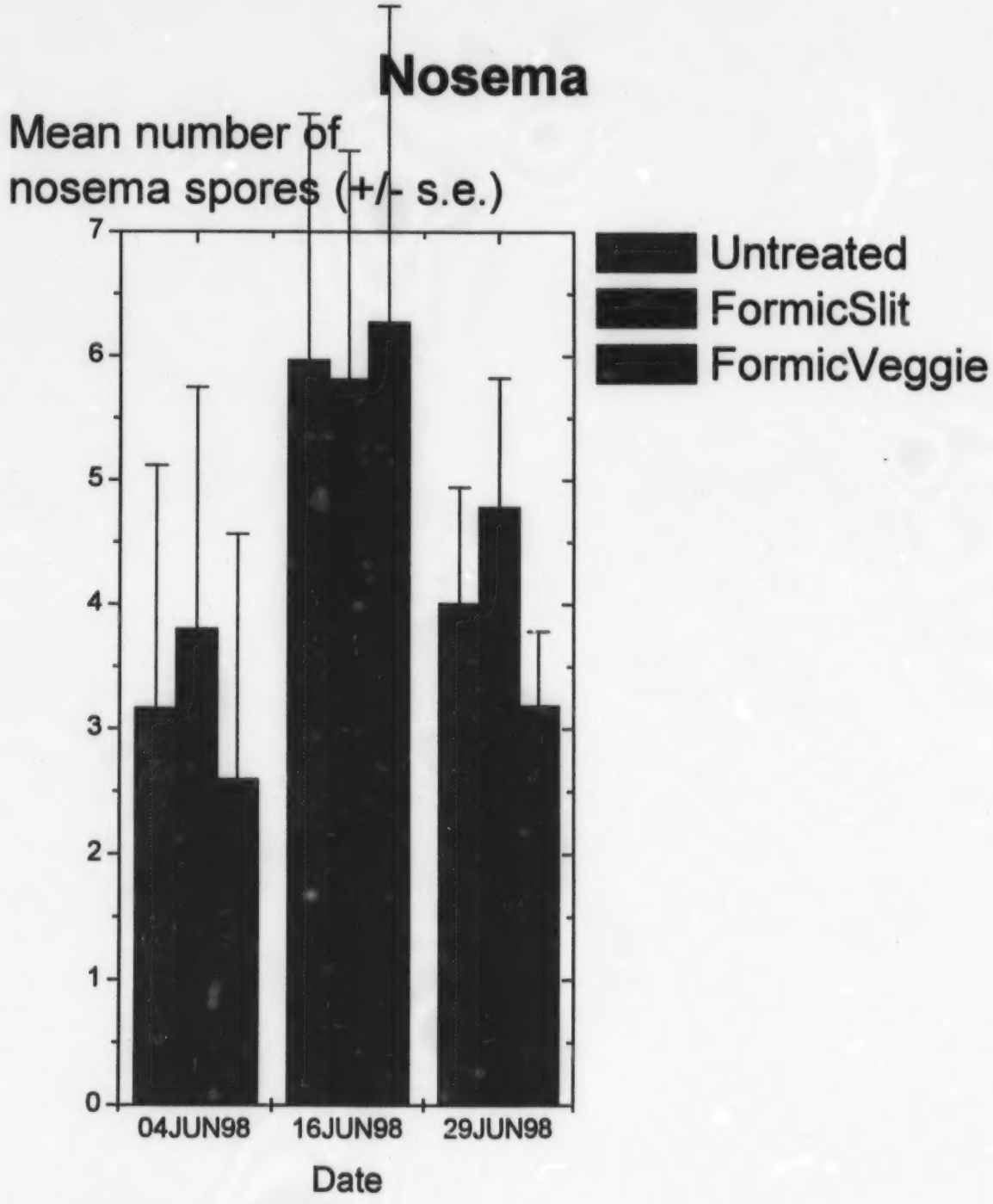

Nosema
Mean number of nosema spores (+/- s.e.)
7
6
5
4
3
2
1
0
04JUN98
16JUN98
29JUN98
Date
Untreated
FormicSlit
FormicVeggie

Figure 20: Effecf of spring slow-release formic acid treatment on nosema in the absence of mites in 1999. FA = formic acid treatment. NoFA= colonies left untreated

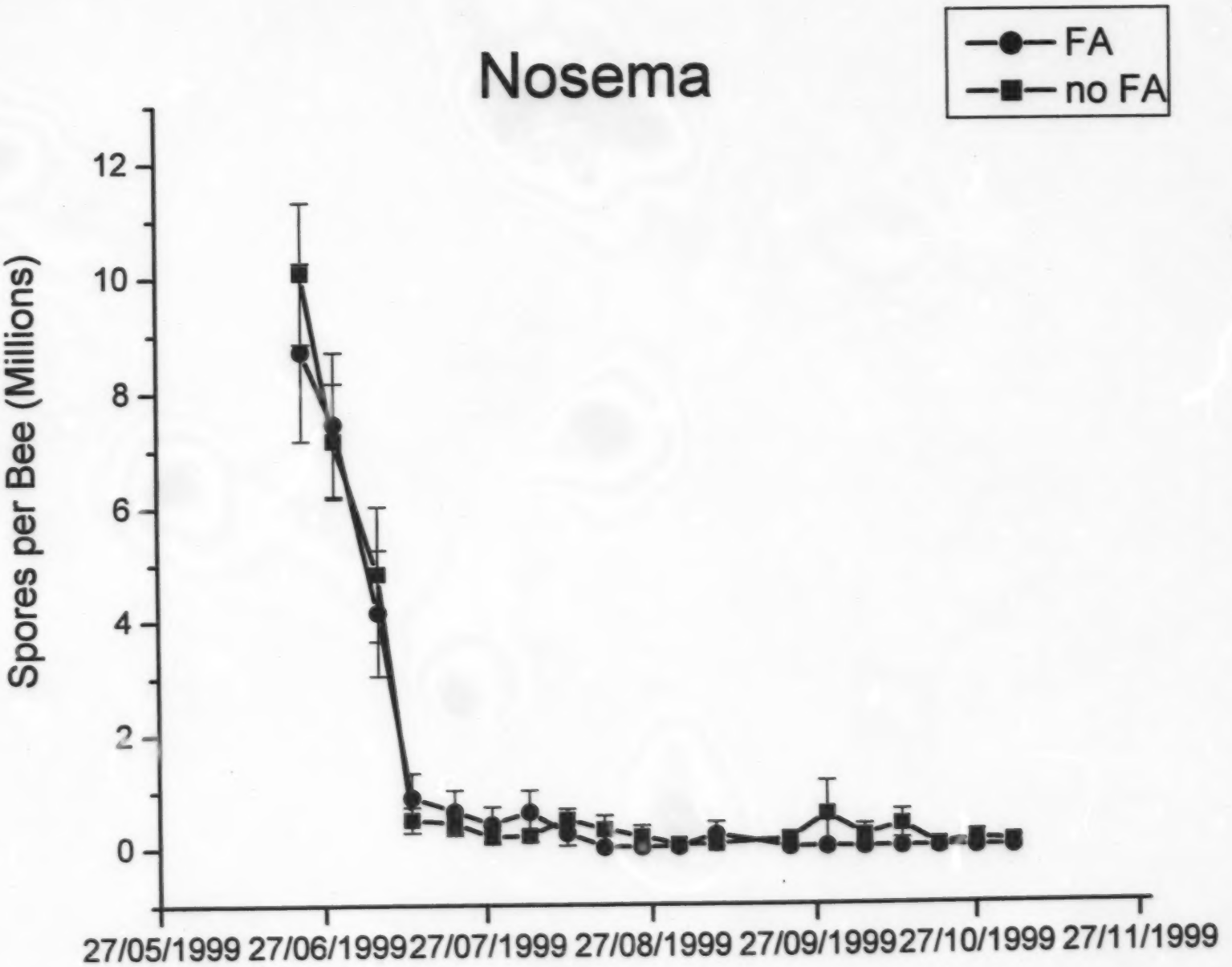

Nosema
FA
no FA
Spores per Bee (Millions)
12
10
8
6
4
2
0
27/05/1999
27/06/1999
27/07/1999
27/08/1999
27/09/1999
27/10/1999
27/11/1999

Figure 21: Effect of spring slow-release formic acid treatment on nosema in the presence of mites in 1999. Varroa = varroa introduced in spring. Novarroa= no varroa present in spring

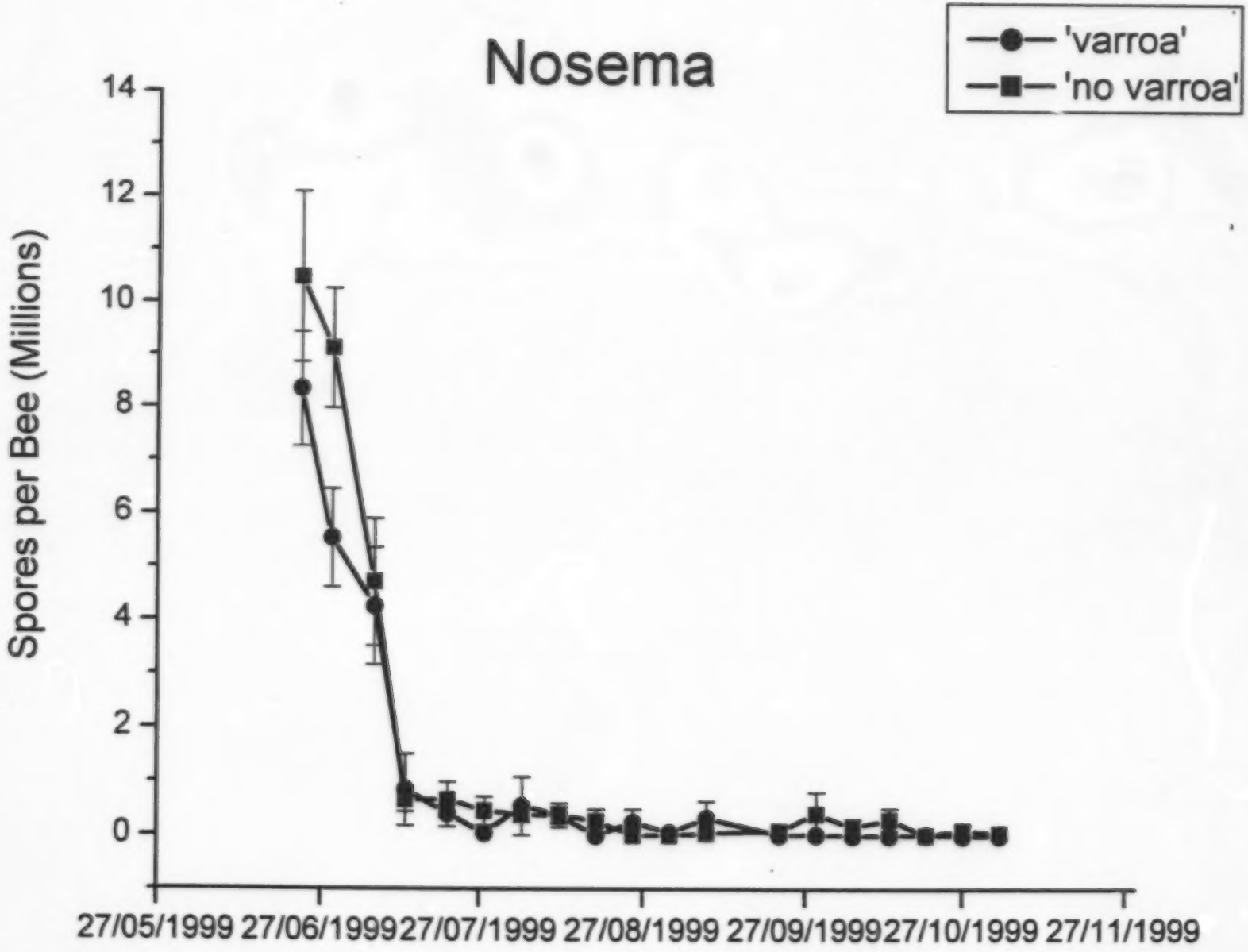

Nosema
'varroa'
'no varroa'
Spores per Bee (Millions)
14
12
10
8
6
4
2
0
27/05/1999
27/06/1999
27/07/1999
27/08/1999
27/09/1999
27/10/1999
27/11/1999

Figure 22: Effect of two types of slow release treatment and no treatment on the mean varroa infestation per hive (mites per 100 bees) in 1998. 04/06/98 = pretreatment levels. 16/06/98 and 29/06/98 = post treatment levels.

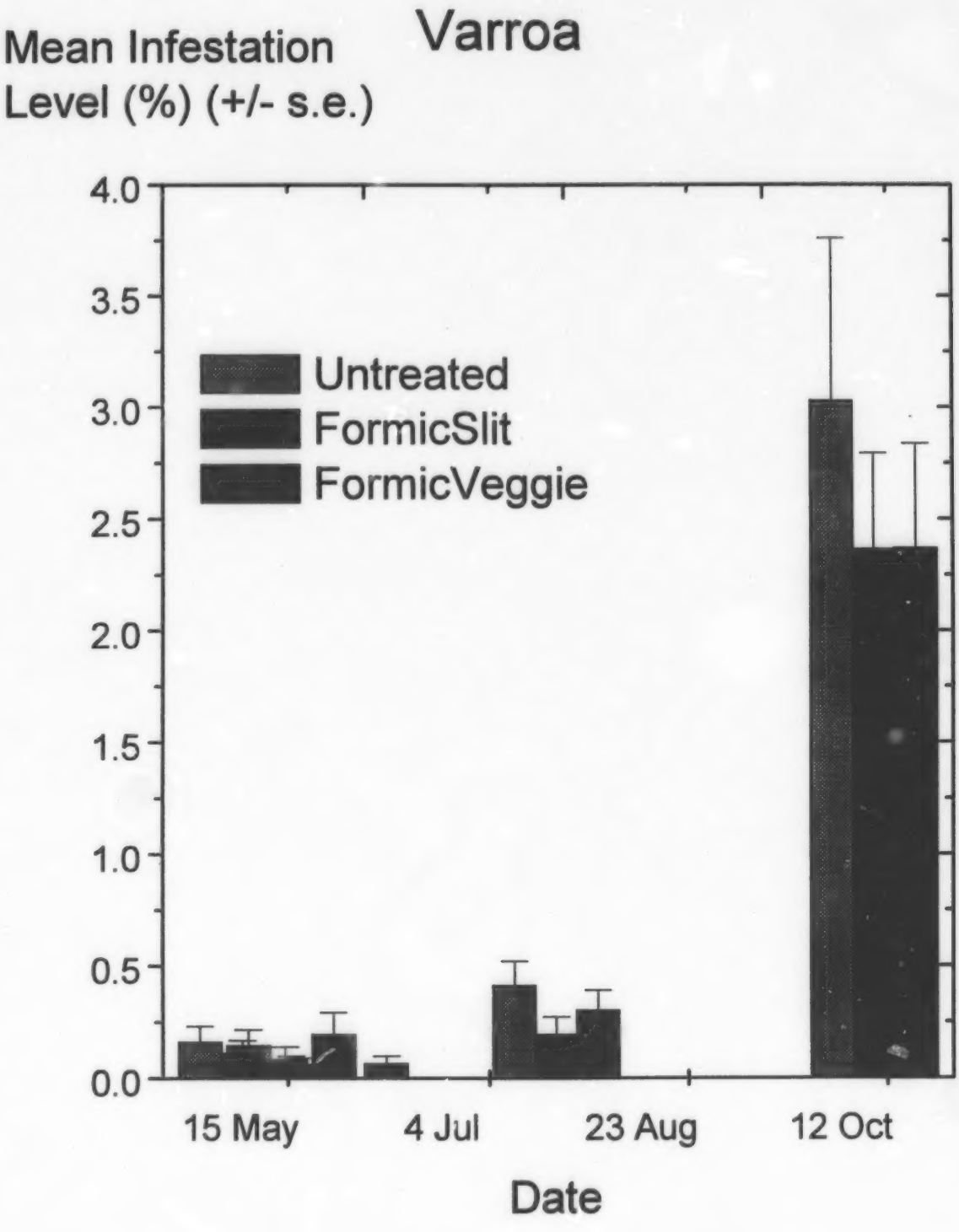
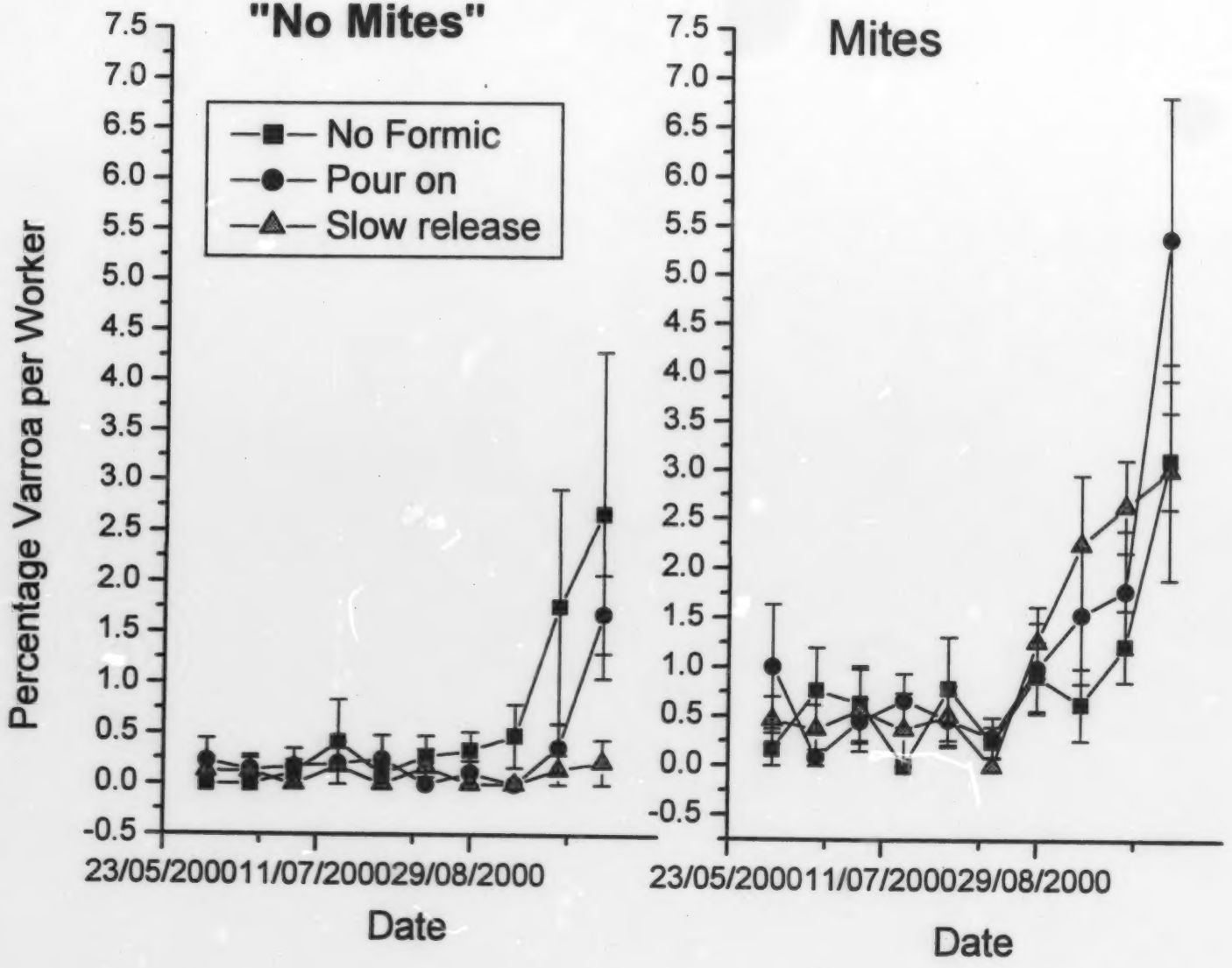

Varroa
Mean Infestation Level (%) (+/- s.e.)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Untreated
FormicSlit
FormicVeggie
15 May
4 Jul
23 Aug
12 Oct
Date

Figure 23: Effect of two types of formic acid treatment and no treatment on the mean percentage of worker bees infested with varroa (mites per 100 bees) in 2000, when mites were present, or absent at the beginning of the season.


"No Mites"
Mites
No Formic
Pour on
Slow release
Percentage Varroa per Worker
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
23/05/2000
11/07/2000
29/08/2000
Date